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Increase in Histamine Synthesis by Liver Macrophages in CCl_4-injured Mast Cell-deficient W/W^v Mice

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ABSTRACT. This study set out to examine the possible role of liver macrophages in histamine synthesis in the injured liver. The effects of the hepatotoxins *Escherichia coli* lipopolysaccharide (LPS) and CCl_4 on histamine synthesis in the liver of mice were evaluated. C3H/HeJ mice were resistant to LPS in including histidine decarboxylase (HDC) in the liver compared with C3H/HeN mice and mast cell-deficient W/W^v mice. However, C3H/HeJ mice did respond strongly to another hepatotoxin, CCl_4, leading to a significant increase in HDC activity. CCl_4 also caused a marked increase in HDC activity and histamine levels in the liver of W/W^v mice. In addition, injection of CCl_4 produced a large increase in the activity of HDC in the spleen and lung of W/W^v mice. HDC activity was confined to the nonparenchymal cells, with parenchymal cells expressing essentially no HDC activity. The CCl_4-induced increase in HDC activity was confined, at least in part, to the liver macrophages. These results indicate that the macrophages are responsible for the increase in HDC-dependent histamine production in the liver caused by the injection of hepatotoxins. The possible role of histamine in liver regeneration after injury is discussed. BIOCHEM PHARMACOL 52;5:809–813, 1996.

KEY WORDS. histamine; carbon tetrachloride; endotoxin; macrophage; histidine decarboxylase; hepatotoxin

Mounting evidence indicates that histamine plays an important role in the pathophysiology of the injured liver. Blood histamine concentration increases in patients with chronic active hepatitis [1] and in rabbits injected with CCl_4, a hepatotoxin [2]. Hepatic histamine content is also elevated markedly in the fibrotic process in rats treated with CCl_4 [3]. Oral administration of HDC† inhibitor to patients with hepatitis relieves the symptoms [1]. In addition, repeated injection of histamine induces liver injury in rabbits [4]. Histamine, at low concentrations, may also participate in the repairing process of the injured liver through the induction of collagen synthesis [5], the proliferation of fibroblasts [6] and by provoking angiogenesis [7]. However, the cells responsible for histamine synthesis in the liver remain obscure. Recently, we demonstrated that mouse peritoneal macrophages produce histamine through HDC [8, 9]. It is well known that a number of macrophage-like cells, including Kupffer cells, reside in the liver. The present study was aimed at increasing our understanding of whether or not liver macrophages also synthesize histamine in the injured liver. CCl_4-induced liver injury was chosen as a model.

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† *Abbreviations:* LPS, lipopolysaccharide; HDC, histidine decarboxylase.

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MATERIALS AND METHODS

Animals and Treatments

WBB6/F1 (W/W^v) mice, which are genetically deficient in mast cells, and C3H/HeN- and LPS-resistant C3H/HeJ mice were raised in the Institute for Laboratory Animal Research, Nagoya University School of Medicine. Male mice at 2–3 months of age were used. In some experiments, the mice were injected intraperitoneally (i.p.) with 0.4 mg/kg of Westphal preparation of LPS from *Escherichia coli* 055:B5 (Difco Laboratories, Detroit, MI, U.S.A.). The control animals were injected with 0.9% NaCl. The other groups of mice were injected with 1600 or 2200 mg/kg of CCl_4, which was dissolved (1:1 v/v) in mineral oil. The control animals received an equal volume of mineral oil. The mice were killed by decapitation 4 hr after injection of the hepatotoxins, unless otherwise noted. Livers, kidneys, spleens, and lungs were removed and homogenized in 0.02 M sodium phosphate buffer (pH 6.2) containing 0.02 mM pyridoxal 5′-phosphate and 0.2 mM dithiothreitol, and centrifuged at 15,000 × *g* for 20 min. The supernatant obtained was used for the assay of histidine decarboxylase (HDC, EC 4.1.1.22) according to the radiometric method described below [10]. A portion of the liver was used for the assay of histamine concentration. It was homogenized in 5 volumes of 0.4 N $HClO_4$. The homogenate was centrifuged at 15,000 × *g* for 20 min at 4°C and the pH of the supernatant obtained was adjusted to between 6.5 and 7.5 with 5 N KOH. The resulting precipitates of $KClO_4$ were re-

moved by centrifugation at 2000 × g for 10 min. The supernatant was used for the assay of histamine [10].

Isolation of Liver Cells

In some experiments, parenchymal and nonparenchymal liver cells, as well as macrophages, were isolated from 2–3-month-old male W/W^v mice and purified by counterflow centrifugation in an elutriation rotor [11–13] with some modifications. Briefly, the mice were injected with 2200 mg/kg CCl_4 4 hr before sacrifice. The liver was first perfused *in situ* with Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBBS)-N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, 10 mM, pH 7.3 containing 0.5 mM ethylenediaminetetraacetate (EDTA) at a rate of 6 mL/min at 37°C for 5 min. The liver was then perfused with Mg^{2+}-free HBSS-HEPES buffer (pH 7.5) containing 0.05% collagenase at 37°C for 15 min. After the perfusion, the liver was excised and the liver cells suspended in HBSS-$NaHCO_3$ buffer. After pipetting, the suspension was filtered through nylon meshes (#60, followed by #150) and the filtrate then centrifuged at 50 × g for 1 min at 4°C. The cell pellet was resuspended in HBSS-$NaHCO_3$ buffer and centrifuged again at 50 × g for 1 min. The parenchymal cells thus obtained were >92% pure as judged morphologically under a microscope. Their viability was approximately 90%, as estimated by Trypan Blue dye exclusion.

The cells in each supernatant fraction were pooled and collected by centrifugation at 500 × g for 7 min (nonparenchymal cells). The cells were resuspended in 5 mL of HBSS-$NaHCO_3$ buffer, which was then loaded into a Hitachi SRR6Y centrifugal elutriator (Hitachi Co. Ltd., Tokyo, Japan). The rotor (Hitachi SRRY-151) speed was 3250 rpm at 4°C; cells were elutriated at 18.5 mL/min, 27.5 mL/min, 32.0 mL/min, and 47.5 mL/min with HBSS-$NaHCO_3$ buffer containing 1% fetal calf serum. The purified liver macrophages were collected at a rate of 47.5 mL/min. The macrophage preparation was estimated to be 90% viable and its purity judged to be >91% after nonspecific esterase staining and 0.81 μm latex bead ingestion (Fig. 5) [14]. The cells were separated by centrifugation at 500 × g for 7 min. The cell pellets were suspended with 0.02 M sodium phosphate buffer (pH 6.2) containing 0.02 mM pyridoxal 5'-phosphate and 0.2 mM dithiothreitol, and were sonicated and assayed for HDC.

Assay for HDC and Histamine

HDC activity and histamine were measured radiometrically by coupling histamine N$^\tau$-methyltransferase to the enzyme [10]. In brief, portions of the sample solutions were incubated in 0.2 M sodium phosphate buffer, pH 6.7, containing 0.05 mM pyridoxal 5'-phosphate, 0.05 mM aminoguanidine sulphate, 0.5 mM dithiothreitol, and 1 mM *l*-histidine hydrochloride (final concentrations). The mixtures were incubated at 37°C for 4 hr and the reaction stopped by heating the reaction mixture in boiling water for 5 min. After centrifugation at 15,000 × g for 5 min, histamine concentration in the supernatant was measured.

Statistical Analysis

Duncan's multiple range test, analysis of variance, or Student's *t*-test was used in each experiment to test the significance of the difference [15].

RESULTS

Effect of Endotoxin and CCl_4 on Histamine Production in the Liver

Figure 1 illustrates the differential effects of LPS (A) and CCl_4 (B) on HDC activity in the liver of C3H/HeN, C3H/HeJ and W/W^v mice, the latter being genetically deficient in mast cells. As shown, LPS injection caused a marked increase in HDC activity in the liver of C3H/HeN and W/W^v mice, but had little effect on C3H/HeJ mice. However, C3H/HeJ mice responded strongly to CCl_4, leading to an increase in HDC activity in the liver. Figure 2 shows the time-courses of the effects of CCl_4 on HDC activity (A) and histamine concentration (B) in the liver of W/W^v mice after injection. CCl_4 injection produced a significant, time-dependent increase in HDC activity in the liver of the mice. However, histamine began to accumulate in the liver 8 hr after CCl_4 injection. CCl_4 injection also produced a large increase in HDC activity in other tissues, such as the spleen and lung, in the W/W^v mice (Fig. 3). The increase

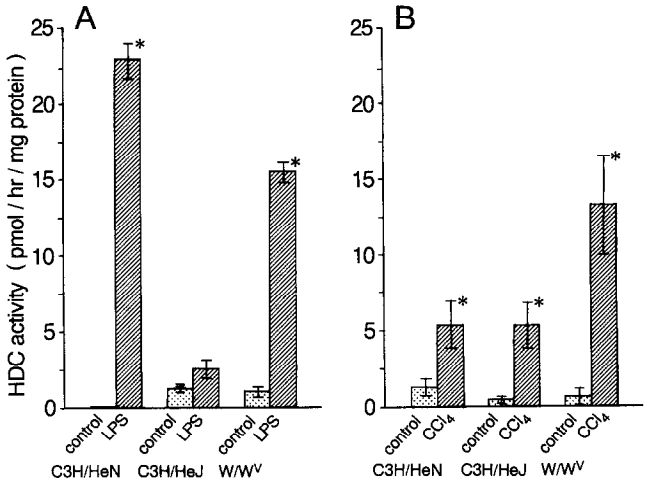

FIG. 1. Differential effects of *E. coli* lipopolysaccharide (LPS) (A) and CCl_4 (B) on histidine decarboxylase (HDC) activity in the liver of C3H/HeN, C3H/HeJ and W/W^v mice. (A) The mice were injected i.p. with 400 μg/kg LPS (LPS) or an equal volume of 0.9% NaCl (control) and killed 4 hr later. (B) The mice were injected i.p. with 1600 mg/kg CCl_4 (CCl_4), which was dissolved (1:1 v/v) in mineral oil, or an equal volume of the vehicle (control) and killed after 4 hr. Values are the mean ± SEM of 3 mice. Statistical difference between the control and the LPS- or CCl_4-injected mice, *$P < 0.05$.

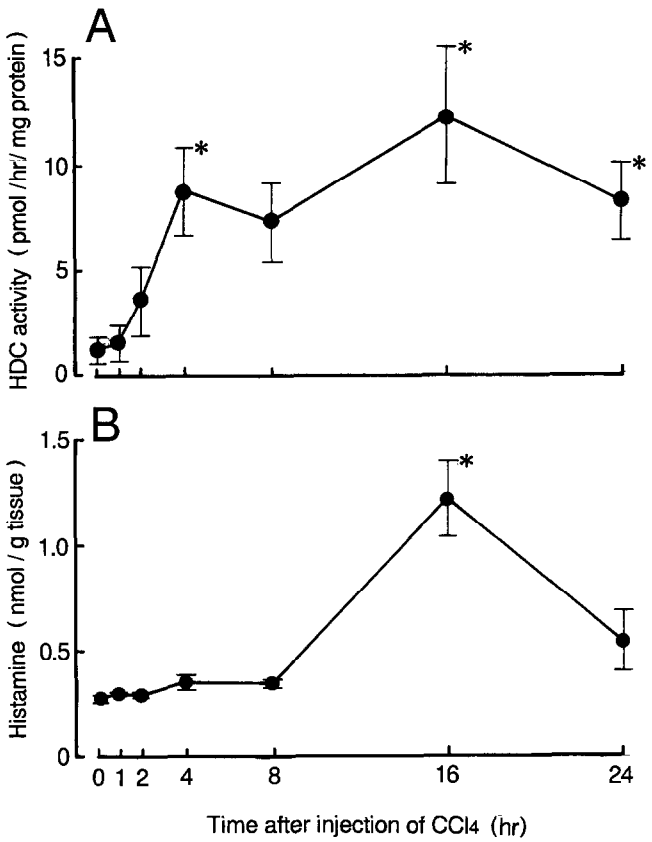

FIG. 2. Time-courses of the effects of CCl_4 injection on HDC activity (A) and histamine level (B) in the liver of W/W^v mice. The mice were injected i.p. with 2200 mg/kg CCl_4 and killed at various time intervals. Values are the mean ± SEM of 3 mice. Statistical difference between the 0-time control mice and the CCl_4-injected mice, *P < 0.05. A lag in histamine synthesis, relative to the increase in HDC, is clearly noted.

in the lung, approximately 18-fold over 4 hr, is particularly striking.

HDC Activity in the Liver

The next study was conducted to determine what type of cells are responsible for the CCl_4-induced increase in HDC activity in the liver. The results obtained showed that HDC activity was virtually confined to nonparenchymal cells, whereas parenchymal cells showed essentially no HDC activity (Fig. 4). We then examined the possible contribution of macrophages to the CCl_4-induced increase in HDC activity in the liver. The majority of the macrophage cells collected in this fraction were positive for nonspecific esterase (data not shown) and ingested Latex beads (Fig. 5), indicative of the involvement of macrophages. The results showed that CCl_4 injection caused a marked increase in HDC activity in the cells of this fraction [control, not detected; CCl_4-injected, 286 ± 38 pmol/hr/mg protein (SEM for 3 separate experiments), $P < 0.01$].

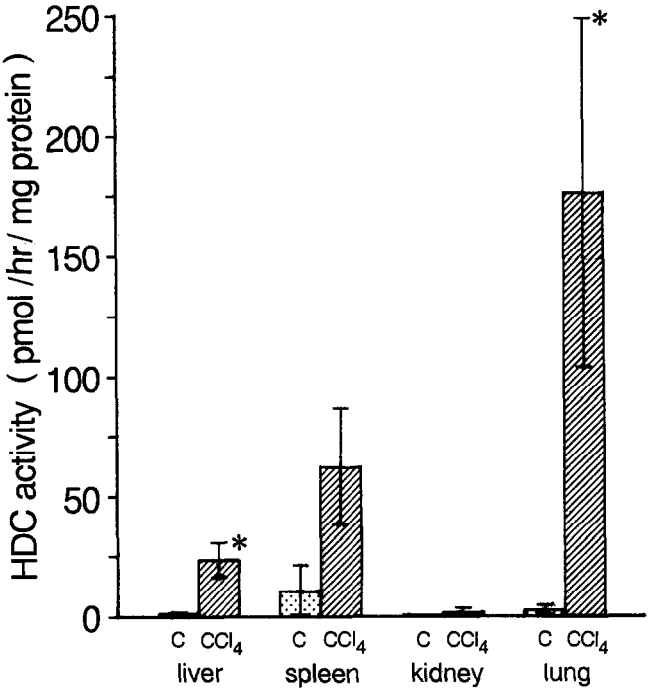

FIG. 3. Effects of CCl_4 injection on HDC activity in the liver, spleen, kidney, and lung of W/W^v mice. The mice were injected i.p. with 2200 mg/kg CCl_4 (CCl_4) or an equal volume of the vehicle (C) and killed after 4 hr. Values are the mean ± SEM of 3 mice. Statistical difference between the control and the CCl_4-injected mice, *P < 0.05.

DISCUSSION

In this study, we examined the possible role of liver macrophages in histamine synthesis in the injured liver. The effects of the hepatotoxins LPS and CCl_4 on synthesis in the liver of mice were evaluated. Our previous studies demonstrated that macrophages of C3H/HeJ mice were far less responsive to LPS in inducing HDC in bone marrow [8]. Because endotoxemia is evident in rats with CCl_4-induced liver injury [16], it is possible that the CCl_4-induced increase in histamine synthesis is mediated by endotoxin. C3H/HeJ mice were used to examine this possibility. The results obtained indicate that this is unlikely, because C3H/HeJ mice did respond strongly to CCl_4, leading to a significant increase in HDC activity in the liver, although they were resistant to LPS. It is also unlikely that mast cells are responsible for increased histamine production in the liver, because it was also evident in W/W^v mice, which are genetically deficient in mast cells. The kinetic studies revealed that HDC activity in the liver began to rise within 2 hr after injection of CCl_4, reached a maximum at approximately 16 hr, and tended to decrease thereafter. In contrast to the HDC activity, the increase in histamine concentration in the tissue became evident as late as 16 hr after the CCl_4 injection. This apparent discrepancy seems to be due to a relatively high level of histamine-degradating enzyme in the tissue [17]. A critical factor permitting us to draw the conclusion that macrophages are responsible, at

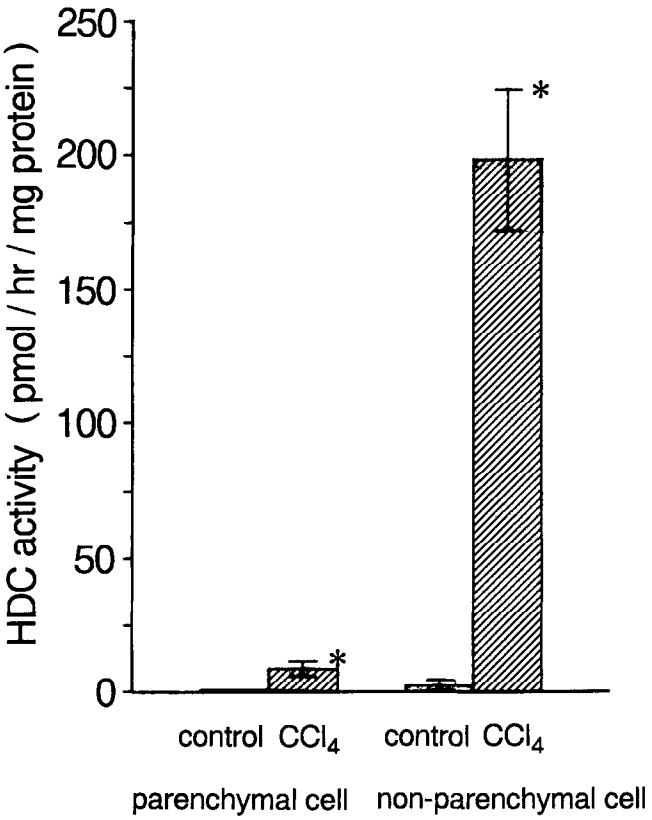

FIG. 4. Effects of CCl_4 injection on HDC activity in parenchymal and nonparenchymal cells of W/W^v mice. The mice were injected i.p. with 2200 mg/kg CCl_4 (CCl_4) or an equal volume of the vehicle (control) 4 hr before sacrifice. The parenchymal and nonparenchymal cells were isolated as described in Materials and Methods. Values are the mean ± SEM of 3 separate experiments. Statistical difference between the control and the CCl_4-injected mice, *$P < 0.01$.

FIG. 5. Mouse liver macrophages in primary culture 24 hr after isolation from the liver of CCl_4-injected W/W^v mice (4 hr after injection), observed by phase contrast microscope. Latex particles (0.81 μm) were added 12 hr before the photograph was taken. All attached cells demonstrated phagostic uptake of Latex particles (×200).

least in part, for increased production of histamine in the injured liver was the use of a macrophage preparation of high purity. An essentially pure population of macrophages was obtained using centrifugal elutriator from the liver of W/W^v mice, which are genetically deficient in mast cells. The majority of the macrophage cells were positive for nonspecific esterase. It remains uncertain, however, whether the LPS- and CCl_4-induced histamine production was due to an increase in the number of liver macrophages, to their activation, or to both. In addition, an increase in the number of liver macrophages may have a dual origin: local replication of resident macrophages and influx of precursor cells such as blood-borne monocytes [18]. Bouwens [18] suggested that the relative importance of these two mechanisms may depend on the experimental or pathologic conditions. When a local stimulus, such as partial hepatectomy or a macrophage-activating drug is applied, local proliferation of resident macrophages, the Kupffer cells, may be preferentially induced. Without a Kupffer cell-specific stimulus as activator of the immune system, recruitment of monocytes may be more important. In our experiments, there seemed to be no appreciable change in the number of macrophages in the liver after treatment of the mice with CCl_4 (data not shown). This suggests that CCl_4-induced histamine synthesis is due to activation of resident macrophages. It remains uncertain whether or not other nonparenchymal cells than macrophages may also participate in histamine production in the injured liver. Ito cells exhibited no HDC activity. Although the endothelial cell fraction had some HDC activity, one cannot exclude the possibility that this was due to contamination of a small number of macrophages in the cell fraction collected using the centrifugal elutriator.

Recent evidence indicates that histamine plays an important role in pathophysiology of the liver. It has been associated with the production of acute-phase proteins in the liver: IL-1, IL-6, or IFN-γ-induced synthesis of complements by hepatocytes is greatly modulated by histamine added to the system [19]. Furthermore, histamine may be involved in certain types of cell proliferation, including regeneration of the injured liver [20]. Synthesis of hepatocyte growth factor (HGF) increases markedly during regeneration of the injured liver, brought about by the administration of CCl_4 or by partial hepatectomy [21, 22]. HGF is produced in liver macrophages [23], and HGF gene expression is consistently stimulated by IL-1 [24]. We have previously shown that histamine acts as an early messenger for the production of IL-1 in peritoneal macrophages [9]. These results, taken together, suggest that CCl_4-induced HGF induction in macrophages is mediated by IL-1 produced by the cells *per se*. Thus, histamine produced by liver macrophages may act as an early messenger in the process.

It is noteworthy that CCl_4 injection also caused a marked increase in HDC activity in the spleen and the lung (Fig. 4). This may be due to the induction of the enzyme in macrophages resident and/or influxed to these immune organs. Nakamura and co-workers [22, 25] have recently shown that CCl_4 injection or partial hepatectomy stimu-

lates HGF mRNA production in the spleen, kidney, and lung. They postulated that these organs can recognize damage of distal organs, such as the liver, and that HGF derived from these immune organs may contribute to tissue repair or regeneration of the injured organs through endocrine-related mechanisms. Histamine may also act as a messenger for HGF synthesis in macrophages in the spleen and lung.

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